A Tunable and Highly-Parallel Picoliter-Dispenser Based on Direct Liquid Displacement

Bas de Heij*, Chris Steinert*, Hermann Sandmaier**, Roland Zengerle*

*IMTEK, University of Freiburg (Germany), Georges-Koehler-Alee 103, D-79110 Freiburg, Germany;
**HSG-IMIT Wilhelm Schickard-Strasse 10, D-78052 Villingen-Schwenningen, Germany
contact: deheij@IMTEK.de

Abstract
We present a new method for the highly parallel and simultaneous delivery of a multitude of reagents in the picoliter range. This method is based on direct displacement of the liquids using an elastomer stamp which simultaneously actuates up to 96 different dosing channels, at a pitch of 500µm. We were able to tune droplet volume from 150 – 720pl and droplet speed from 0,2m/s – 2,8m/s using printheads with 50µm nozzles. In contrast to all other inkjet techniques the new direct displacement method enables the precise control of dispensing quantity in the picoliter range regardless of reagent viscosity.

Introduction

Microarrays are a certain type of biochips that enable fast and highly parallel analysis of biomolecules. It is expected that the microarrays will be the main analysis and diagnosis tools for the coming decades. Crucial for high throughput production of microarrays is a fast method for dispensing 100 – 10.000 different reagents in picoliter droplets, at a pitch of 200 – 500µm. In the past we presented a printhead with 96 channels at a pitch of 500µm, firing simultaneously by pneumatic actuation [1, 2]. Based on this technology a fully automated production line with an up to now unmatched throughput was built (fig.1). It was seen that with the pneumatic actuation reagents having different viscosities are dispensed with different volumes and that a controlled adaptation of droplet volume and droplet speed is limited. In this paper we present for the first time a new actuation method based on direct liquid displacement that overcomes these limitations.

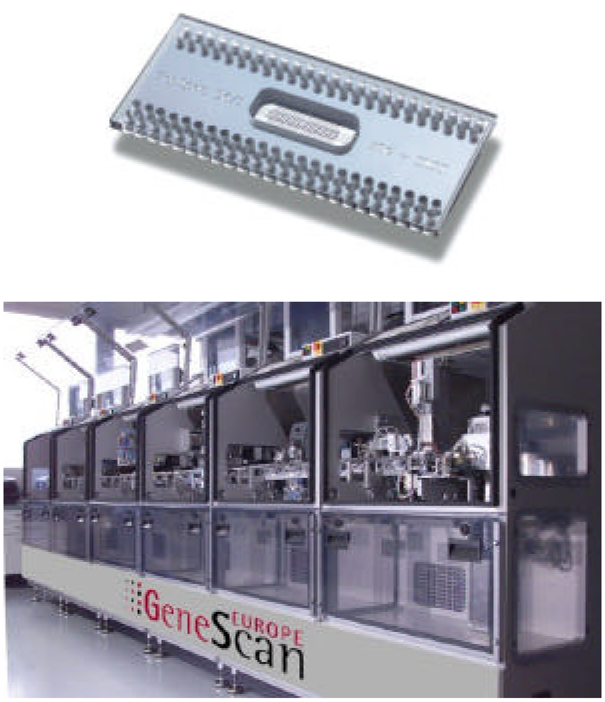


Fig. 1: Production line based on TopSpot technology, spotting up to 1.440 different reagents at a throughput of 300 biochips / hour, operated at Genescan Europe AG, Freiburg (D) Top: the 96 channel printhead.

The TopSpot print technology is based on a silicon micromachined (mainly DRIE) printhead (fig. 1) sandwiched between two glass layers. The spotting solution is filled in the reservoir (see fig. 2). Capillary forces will draw the liquid to the nozzles in the centre print window. A piston is placed in the print window defining a certain air chamber. When actuated by the piezo actuator the piston will compress the air in the chamber causing a pressure pulse which will be transferred into the liquid. This will eject the droplets.

Experimental

In the previous concept the pneumatic pressure was generated by a fast mechanical displacement of a piston, reducing the volume of the actuation chamber. Due to the compressibility of the air a controlled tuning of the dispensed liquid volume

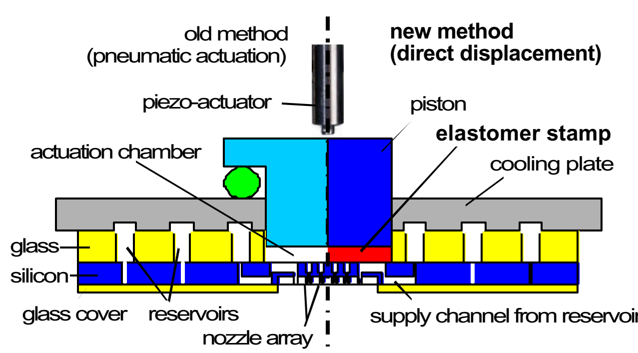


Fig. 2: Schematic of the old method based on air compression (left) and the new method based on direct displacement (right).

turned out to be difficult. In the new, direct displacement method, the compressible air is replaced by an incompressible but deformable medium (e.g. rubber) (fig. 2). The movement of the piston forces the elastomer into the channels of the printhead displacing the reagents there (fig. 3). The dispensed volume is defined by the amplitude of the piston movement, which is controlled at nanometer accuracy by a piezo-stack actuator. For maximum accuracy the precision of the micromachined printhead is needed. The incompressibility of the elastomer as well as the high stiffness of the actuator eliminates any feedback from liquid viscosity on the actuator stroke.

Different kinds of elastomers (Shore hardness) and different piston geometries were investigated with

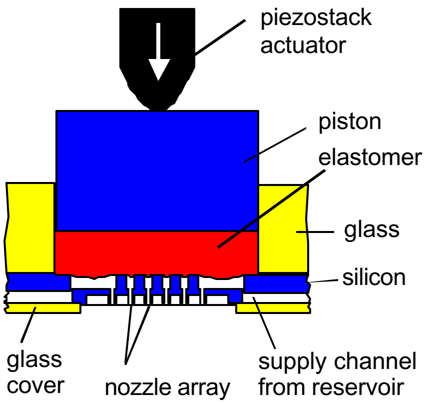


Fig. 3: Schematic of the elastomer deformation into printhead channels displacing the reagents

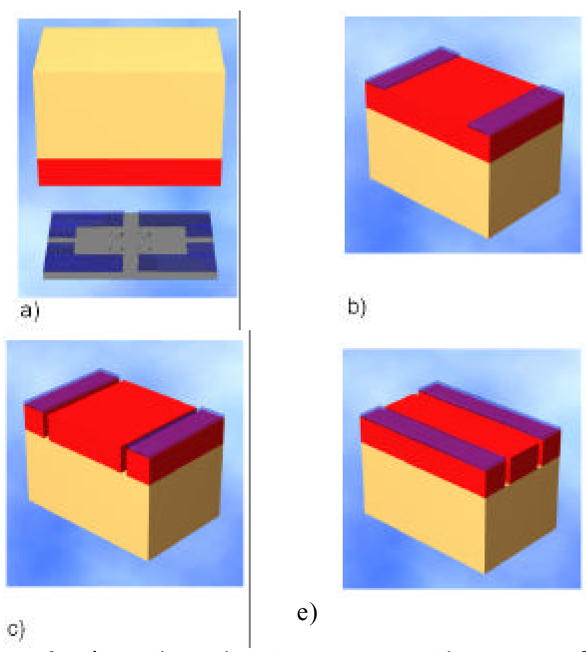


Fig. 4: Investigated stamp geometries a: flat elastomer stamp with a spacer on the printhead. b. spacer directly adjacent to the rubber stamp. c: Spacer with spacing d: spacer with spacing along the longer side of the stamp.

respect to risk of cross-contamination and the ejection performance. First experiments concentrated on finding an optimal procedure for placing the rubber stamp in the printhead filled with reagents. We investigated different stamp geometries (fig. 4) that guarantee a parallel approach between the elastomer and printhead surface. This can be achieved by placing a spacer structure on the printhead (type a) or on the elastomer stamp (types b..e). Different elastomer hardness was tried for the spacer structure. The elastomer for the actual liquid displacement was in all cases SBR40. This spacers in combination with the hydrophobic elastomer surface eliminates cross contamination which otherwise could occur due to capillary effects, just before the stamp touches the printhead.

Secondly the droplet ejection performance was investigated. Goal was to find the relation between actuator dynamics (stroke and speed) and the ejected droplets (volume and speed). In these experiments we focussed on finding the parameter window for which stable and satellite free droplet ejection is possible

Results and Discussion

The supply channels are open where they lead to the nozzles (see Fig 5). When placing the rubber stamp it can happen that fluid is drawn in the print window, causing cross contamination. One solution to this is to guarantee a complete parallel approach of the two surfaces. This can for instance be achieved by using special stamp geometries as shown in fig 4. The outside rim of the stamp will first touch the printhead inside, where there are no supply channels, and so aligns the rest of the surface. The effect of different stamp geometries at the same actuator settings will be discussed later.

The cross contamination was checked by filling the printhead with two different dyes in a checkerboard

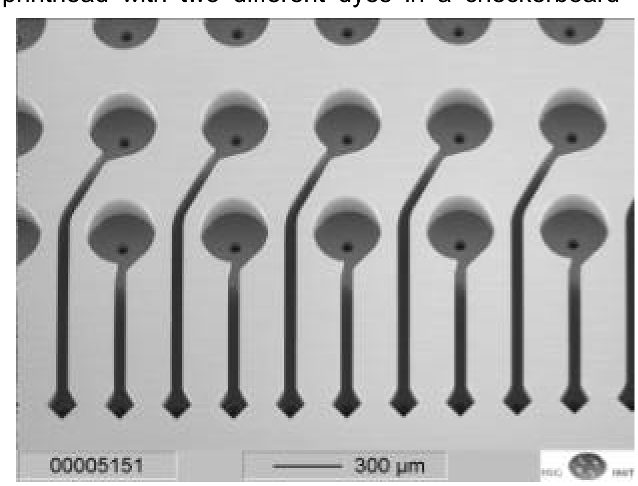


Fig. 5 The open connection channels from the via to the nozzle.

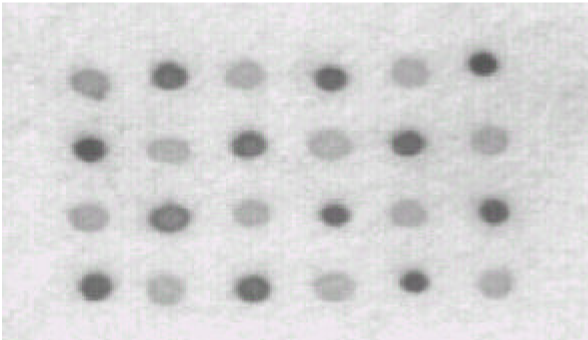


Fig. 6: Two different colours are printed from neighbouring channels to demonstrate the absence of cross contamination.

pattern (see fig. 6), any cross contamination would result in a colour mixing. Non of the proposed shapes showed any colour mixing, while the original flat one clearly showed problems.

The elastomer behaves like an incompressible medium and flows like a liquid for small deformations. For better understanding of the functioning of the direct displacement principle one could thus think of a direct fluidic contact between the actual piston and the ejection nozzles. To test this we measured the ejected droplet volume as function of the piston stroke. A clear linear relation was found here. The measurement results are shown in Fig 7. Different actuation speeds (same stroke in shorter time) were tried as well as two different stamp designs. First thing to notice is the zero crossing of the interpolation line and the very low deviation of the measurement points from this line. Secondly can be seen that in this case there is no influence of the stamp design and the actuation speed on the droplet volume.

From the direct coupling of the piston and the droplet ejection it is also expected that the ejection speed is directly proportional to the piston speed. Several experiments were performed, where a certain piezo stroke was made in different time periods. The result is shown in Fig 8. First thing to note here is again the linear relationship between the stroke speed and the droplet speed. Secondly it can be seen that larger droplets (larger stroke) tend to fly slower. A minimum actuator speed is necessary for droplets to break clear of the nozzle, causing an offset in the velocity scale. This can be explained by the energy that is used to build the droplet's outer surface and the force it takes for the droplet to break clear of the nozzle.

Whereas shape a and b did not show a significant difference in the ejected droplets, a far more profound difference can be found for shapes c and e (as shown in fig. 9). The outer, elevated, rim was made in a harder rubber as the rest of the stamp. This causes for the shape e, with the larger area of harder rubber, a higher counter force during actuation as with shape c. This force also works on the printhead itself, and it is expected that this force bends the entire printhead and so effectively lowers the actuation impulse.

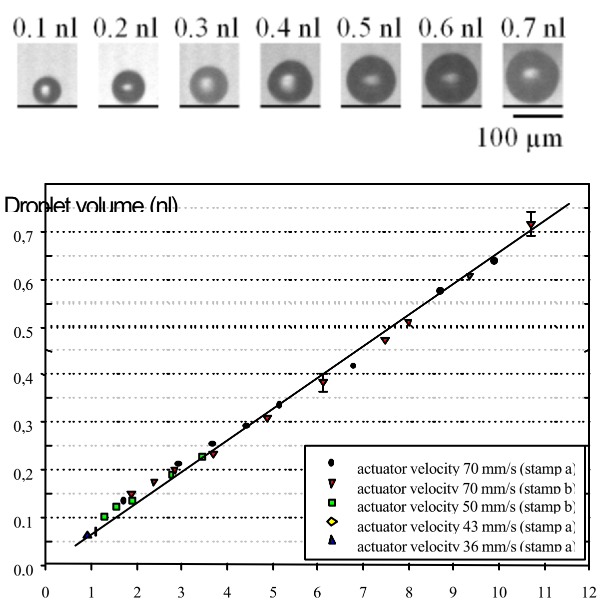


Fig.7 The ejected droplet volume (nl) versus piezo stroke (µm). Measurements with stamp (a) and (b).

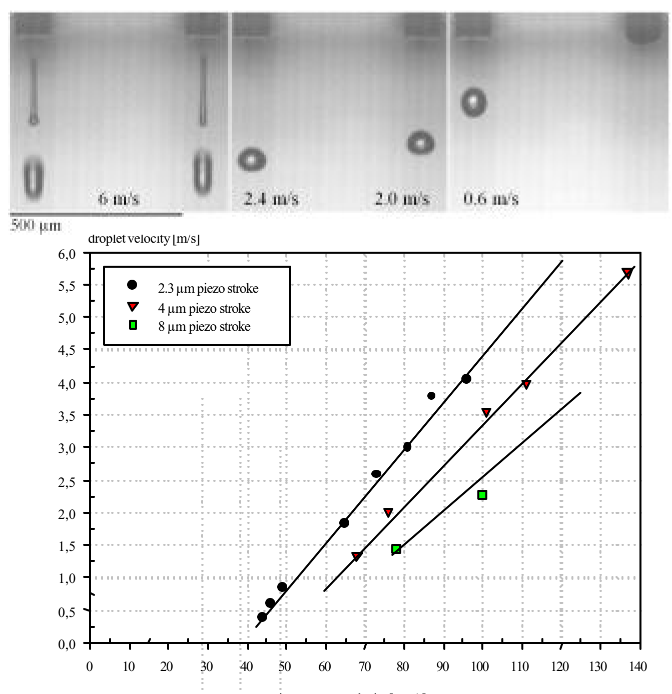
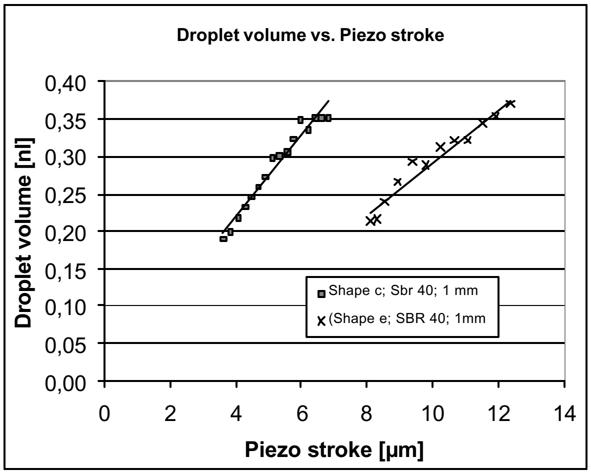


Fig. 8 Influence of the piezo speed (mm/s) on the droplet speed.(m/s), for stamp (a)



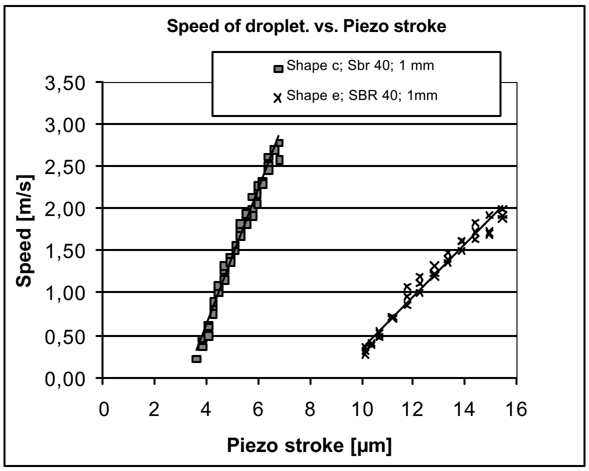


Fig. 9 Droplet volume and droplet speed versus piezo stroke for two different stamp designs (c and e).

An other factor in this is the amount of free space between the stamp and the print window at the beginning of the actuation. With more space available to deform laterally, less elastomer will enter the nozzles.

Conclusion

Figure 7 shows that the droplet volume can be precisely controlled by the piezo-stack actuation over a range of 150pl to 720pl. Droplets as small as 60pl were also generated with a different stamp design. The new concept has a clear linear relation between actuation amplitude and dispensed volume.

It can also be seen in fig. 7 that the sensitivity of the volume variation can be adapted by using different stamps (geometries and hardness).

It was shown that it is possible to place the rubber stamp in the printhead without causing cross contamination. The designs (a) and (b) showed equal performance. The design (b) is favourite for the application for practical reasons (only one part, that can be build as an disposable).

The designs (c) and (e) showed that it is possible to tune the sensitivity of the piezo stroke versus the droplet size. This was mainly caused by the space between the spacer bars and the actual stamp, permitting for a lateral expansion.

The two parameters, piezo stroke and piezo speed are not fully independent. For smaller droplets to break clear, a higher speed is necessary. The same speed might result in satellite droplets for larger strokes. This means that for every desired droplet size there is a limited set of parameters that work problem free.

The complete control of the droplet volume signifies that we can control the actual spotsize in the microarray. The first results also showed that this droplet volume (and thus the spotsize) is independent of the dispensed liquid. This significantly enhances the flexibility in microarray production.

Acknowledgements

The practical work of Chris Steinert is highly acknowledged. Also important to notice is the work of NMI (Reutlingen, Germany) on the hydrophobic coating of the nozzle surfaces.

References

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